cMyBP-C is altered during heart failure (HF). There are several identified phosphorylated residues, including the regulatory phosphorylation sites Ser275, Ser284 and Ser304. Methods: Comparison of the extent of MyBP-C phosphorylation status in failing (N=10) and non-failing (N=10) human left ventricle (LV) tissue. The myofilament subproteome was extracted using the 'IN Sequence' method prior to analysis with some samples dephosphorylated using alkaline phosphatase as control. Sample analysis was carried out using gel- (e.g. phos-tag) and mass spectrometry (MS)-based methods. Results: Using an MS-based phospho-peptide ($Ti0_2$ chromatography) enrichment strategy we identified novel phosphorylation sites at residues Ser286 and Thr290, located in the M-motif. These sites were as well observed in MyBP-C from LV canine hearts. A novel site at residue Thr1109, the titin binding domain C9, was only observed in the samples without phospho-enrichment. Our results indicate a marked reduction in cMyBP-C phosphorylation at residues Ser284, Ser286, Thr290 and Thr1109 in end-stage HF compared to the non-failing group. Conclusion: This study provides evidence for novel phosphorylation sites on cMyBP-C both in human and canine hearts and reduced phosphorylation levels in the end-stage failing heart.

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Effects of HCM Missense Mutations in the M Domain of Cardiac Myosin Binding Protein C on Calcium Sensitivity of Force and Rate in Rat Trabeculae

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Recombinant N-terminal domains of cardiac myosin binding protein C (cMyBP-C) increase calcium-sensitivity of force and the rate of tension redevelopement (ktr) when added to permeabilized rat trabeculae. We previously demonstrated that the regulatory domain of cMyBP-C, referred to as the MyBP-C motif (or ''M domain''), is required for these effects. Here we investigated the effects of single amino acid missense substitutions within the M-domain that are associated with human hypertrophic cardiomyopathy (HCM) on force development and ktr in permeabilized trabeculae from rat right ventricles. Individual substitutions (R322Q, E330K, V338D, and L348P) were introduced into a recombinant mouse C1C2 protein (encompassing domains C1-M-C2 of murine cMyBP-C) by site directed mutagenesis and effects were compared to wild-type C1C2. All four of the mutations affected the ability of C1C2 to augment force. Whereas 5 μM wild-type C1C2 induced a pronounced leftward shift in Ca2+ sensitivity of tension (~0.5 pCa units) and increased ktr at all submaximal Ca2+ concentrations, 3 of the mutations reduced the effects of C1C2. Another variant increased the efficacy of C1C2 and increased passive force independent of Ca2+, but reduced maximal Ca2+ activated force. Together these results indicate that cMyBP-C variants associated with HCM could directly disrupt sarcomere contractile properties through gain or loss of functional effects and that at least some cMyBP-C missense mutations may cause disease through a poison polypeptide mechanism. This work is supported by NIH HL080367 to SPH and a DOD NDSEG graduate fellowship to KLB.

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Common Mechanical Properties of Recombinant and Native Cardiac Myosin Binding Protein-C by Atomic Force Microscope

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Cardiac myosin binding protein-C (cMyBP-C) is a member of the immunoglobulin (Ig) superfamily of proteins and consists of 8 Ig- and 3 fibronectin (Fn)-like domains along with a unique regulatory sequence referred to as the MyBP-C ''motif'' or M-domain. Previously we used atomic force microscopy (AFM) to investigate the mechanical properties of the different domains of murine cMyBP-C expressed using a baculovirus/insect cell expression system. To investigate whether the mechanical properties of cMyBP-C are conserved across species, here we used AFM to investigate the mechanical properties of human recombinant cMyBP-C expressed using a baculovirus/insect cell expression system and native cMyBP-C purified from bovine heart. AFM force-extension spectra were obtained from cMyBP-C molecules by randomly adhering individual molecules to the tip of an AFM cantilever and moving the cantilever to impose a load that stretched the molecules. Results show that the spectra for the human recombinant and bovine native proteins are remarkably similar with the first Ig/Fn-like domain unfolding events occurring at low (~50 pN) forces and the highest stability domains unfolding at ~190 pN. Experiments also revealed frequent unfolding events that appeared coupled such that lower stability domains would often unfold after higher stability domains. These unexpected force ''drops'' were highly reproducible and occurred in spectra from both human cMyBP-C and bovine cMyBP-C. In addition, both recombinant and native cMyBP-C exhibited an ~100 nm long extensible region that could be stretched with less than 50 pN force prior to the unfolding of Ig and Fn-like domains. Combined with our previous results from mouse cMyBP-C, these results establish common mechanical features of cMyBP-C across species. Supported by NIH HL080367.

2836-Pos Board B606

The Effects of C-Terminal Mutations on the Folding of Cardiac Myosin Binding Protein-C

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Cardiac myosin binding protein-C (cMyBP-C) is a modular protein involved in stabilizing interactions with the thick filament of the sarcomere. The N-terminus of cMyBP-C associates with actin and myosin S2 and the C-terminus interacts with titin and myosin rods. While no high-resolution structure of C-terminal cMyBP-C exists, disruption of this region is proposed to destabilize cMyBP-C and adversely affect cardiac structure and function. In particular, deletion of 25 base pairs (Δ25) in the gene encoding for cMyBP-C results in amino acid substitutions in the C10 domain of cMyBP-C (C10 Δ25) which may be associated with the development of hypertrophic and dilated cardiomyopathies by unknown molecular mechanisms. The prevalence of this mutation is approximately 1% of the world population, underscoring the necessity of determining its role(s) in the pathogenesis of cardiomyopathies. In this study, circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopies have been used to examine the conformation of wild-type (Wt) and mutant C-terminal domains of cMyBP-C. Comparison of near UV CD spectra revealed alterations in the packing of aromatic residues in C10 Δ25 suggesting it is less stably folded as compared to C10 Wt. C10 Δ25 exhibited less beta-sheet content than C10 Wt as evidenced by the estimation of secondary structure from CD data. NMR analyses of amide proton/nitrogen chemical shifts and linewidths were used to probe the conformation of C10 domains and to map residues of importance in protein-protein association onto cMyBP-C models. Taken together, these data suggest that the Δ25 mutation structurally modulates cMyBP-C sites involved in binding titin and myosin.

2837-Pos Board B607

Human Signaling Scaffold Protein (mAKAP) Binding Kinetics to PKA and Phosphodiesterase (PDE4DE): Implications for a Possible Role in Heart Failure

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Heart failure is a leading cause of morbidity and mortality in the USA. There are several therapeutic agents available for heart failure management. In particular, agents that block beta-adrenergic receptor improve mortality rate among heart failure patients by enhancing cardiac function. Beta-adrenergic receptor stimulation signals through protein kinase A (PKA) dependent phosphorylation, partly by binding to A-kinase anchoring proteins, influencing calcium homeostasis. In particular, mAKAP (muscle-selective A-kinase anchoring protein) is targeted to specific intracellular compartments resulting in localization of PKA with its substrates as well as to bind with ryanodine receptors and phosphodiesterase-4D3 (PDE4DE). The signal transduction complex formed by the scaffold protein mAKAP at the perinuclear envelop of striated myocytes contains cAMP specific binding protein PDE4D3 which is responsible for cAMP signaling termination. Agents that modify PKA signaling would be expected to mediate an altered inotropic response. From different genomic databases, we have recently identified fifteen human mAKAP coding nonsynonymous polymorphisms located within or near key protein binding sites critical to beta-adrenergic receptors signaling. Seven of these mutants were cloned for the purpose of comparing whether those substitution disrupt mAKAP binding to either the PKA binding domain R2alpha or the phosphodiesterase PDE4DE. Using surface plasmon resonance (Biacore 2000) we demonstrate specific binding of wild type mAKAP to PDE4DE. Experiments were run in triplicate and as twofold serial dilutions to explore the kinetics of the interaction and analyzed using Scrubber2 with a 1:1 Langmuir model. Comparative analysis of the binding responses of mutations to mAKAP could provide important information about how these mutations modulate signaling.

2838-Pos Board B608

Single Molecule Studies of a Titin Mutation Linked to Cardiac Disease

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Recently, a mutation in the 10th immunoglobulin(Ig)-like domain in titin's elastic I-band was found in a family affected with arrhythmogenic